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*Review*

# Breast Cancer Metastasis to the Central Nervous System

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**Clinically symptomatic metastases to the central nervous system (CNS) occur in ~10 to 15% of patients with metastatic beast cancer. CNS metastases are traditionally viewed as a late complication of systemic disease, for which few effective treatment options exist. Recently, patients with Her-2-positive breast tumors who were treated with trastuzumab have been reported to develop CNS metastases at higher rates, often while responding favorably to treatment. The blood:brain barrier and the unique brain microenvironment are hypothesized to promote distinct molecular features in CNS metastases that may require tailored therapeutic approaches. New research approaches using cell lines that reliably and preferentially metastasize *in vivo* to the brain have been reported. Using such model systems, as well as *in vitro* analogs of blood-brain barrier penetration and tissue-based studies, new molecular leads into this disease are unfolding. *(Am J Pathol 2005, 167:913–920)***

## Natural History of CNS Metastasis

Of the nearly 1.3 million people diagnosed with cancer in the United States each year, ~100,000 to 170,000 will develop brain metastases, for an annual incidence of ~4.1 to 11.1 per 100,000 population (American Cancer Society Cancer Facts and Figures 2005, available at *http://www.cancer.org*).[1] Large autopsy studies suggest that between 20% and 40% of all patients with metastatic cancer will have brain metastases (*http://www.cancer.org*).[1–4] Given their overall greater frequency, lung and breast cancer are by far the most common tumors to present with brain metastases.[2,4] The incidence of symptomatic brain metastases among women with metastatic breast cancer ranges from 10 to 16%.[5] On average, the median latency between the initial diagnosis of breast cancer and the onset of brain metastasis is ~2 to 3 years.[1,2] In most cases, breast cancer patients develop brain metastases after metastases have appeared systemically in the lung, liver, and/or bone.[6] For the purposes of this review, central nervous system (CNS) and brain are used interchangeably.

Several risk factors for brain metastases have been reported. Young age appears to correlate with elevated risk.[5,7] In a study of 1015 women with metastatic breast cancer, brain metastases occurred in 9% of women with estrogen receptor-negative (ER−) primary tumors, compared to 5% of patients with ER+ primary tumors.[8] Many human breast cancers (25 to 33%) express Her-2, also known as the epidermal growth factor receptor erbB2 or the *neu* oncogene [Online Mendelian Inheritance in Man (OMIM) accession number 164870; *http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id = 164870*, accessed 2.25.05]. Amplification or overexpression of Her-2 correlates with a shorter disease-free and overall survival time[9] and also appears to associate with a higher incidence of brain metastases.[10–12]

The metastasis of breast cancer to the CNS, either the brain parenchyma or the leptomeninges, is generally a late feature of metastatic disease. Metastases to the brain parenchyma are thought to be hematogenous in origin. In a retrospective survey of breast cancer patients with brain metastases, 78% had multiple intracerebral metastases, 14% had a solitary intracerebral metastasis, and the remaining 8% had leptomeningeal metastases.[7] Breast cancer is the most common solid tumor to exhibit leptomeningeal colonization.[13] Within the three membranous coverings, or meninges, that surround the brain, leptomeningeal metastases arise on the innermost covering (pia) and the middle membrane (arachnoid) or in the cerebral spinal fluid (CSF)-filled space between the arachnoid and the pia (subarachnoid space).[4] Spread to the leptomeninges may occur via multiple routes including hematogenous, direct extension, transport through

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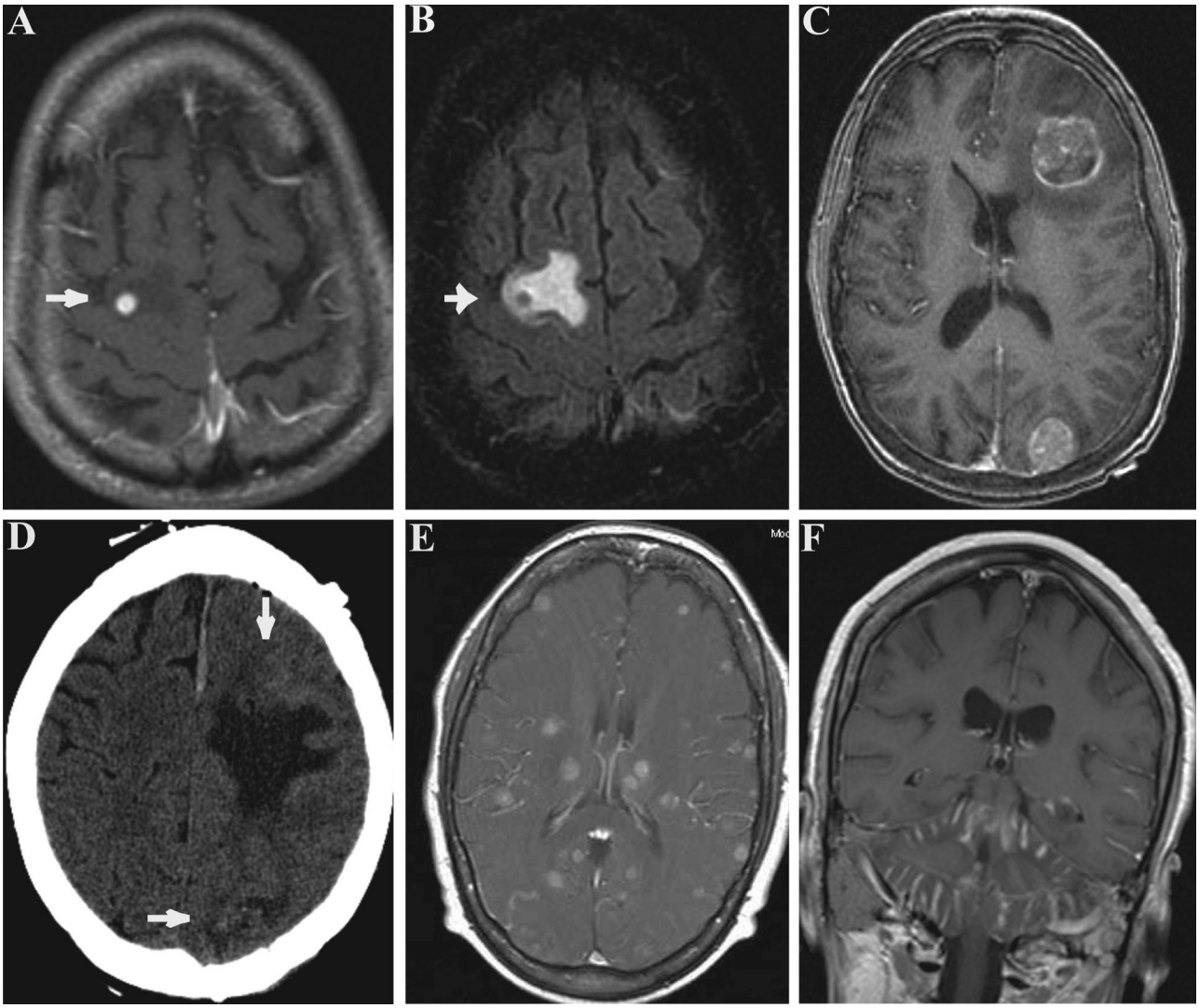


**Figure 1.** Representative manifestations of breast cancer metastasis to the brain. **A:** Solitary metastasis (~1 cm in size) to the brain demonstrated by MRI (T1-weighted, axial, postgadolinium image). The tumor is in the posterior right frontal lobe. **B:** The tumor (**arrow**) is surrounded by a significant amount of peritumoral edema (T2-weighted axial MR image). **C** and **D:** Multiple metastasis to the brain before (**C**) [demonstrated by MRI (T1-weighted images with the administration of gadolinium for contrast)] and after (**D**) surgical resection (axial computed-tomography scan with contrast; **arrows** in **D** indicate the location of the resection). The tumor in the left frontal region was intraparenchymal and the tumor in the left parieto-occipital region was dural-based. **E:** Miliary metastases demonstrate multiple, contrast-enhancing lesions on a single T1-weighted, gadolinium-enhanced MRI slice, ranging in size from 2 to 3 mm to 1 cm. **F:** Carcinomatous meningitis. Multiple linear enhancing tongues of tumor can be seen outlining the cerebellar folia on theT1-weighted gadolinium-enhanced MRI.

the venous plexus, and extension along nerves or perineural lymphatics.[13] Once the tumor cells reach the leptomeninges, they are thought to spread via the CSF (Figure 1).

Diagnosis of brain metastases is based on patient symptoms and neuroimaging. The most common clinical symptoms of parenchymal brain metastases include headaches and alterations in cognition, mental status, and behavior. Frequent signs that generally reflect the location of the tumor and the influence of peri-tumoral cerebral edema include nausea and vomiting, seizures, and deficits in sensation, motor function, speech, and/or vision. Lesions in the cerebellum and brain stem, which are less common than those in the cerebral hemispheres, can cause ataxia, cranial neuropathies, and upper motor neuron dysfunction, as well as additional signs and symptoms related to hydrocephalus, such as headache, memory loss, or behavioral problems. Contrast-enhanced neuroimaging, ie, computed-tomography or magnetic resonance imaging (MRI), is the mainstay of diagnostic evaluation. Ancillary studies, such as lumbar puncture or positron emission tomography, may be indicated in some situations in which symptoms and signs such as headache, cranial neuropathy, or alterations in cognition suggest leptomeningeal carcinomatosis rather than a parenchymal mass.[3]

Breast cancer involving the CNS is traditionally viewed as a late complication of progressive metastatic disease, for which few effective treatment options exist. For all brain metastatic patients, those with controlled extracranial tumor, age less than 65 years, and a favorable general performance (Karnofsky performance status $\geq$70) fare best whereas older patients with a Karnofsky performance status $<$70 do poorly.[14,15] Patients with sol-

itary metastases and with a longer disease-free interval also tend to fare well.[14,15] Treatment strategies have been reviewed in several recent monographs.[15,16] Many of the randomized studies cited pertain to patients with brain metastases from multiple cancer histologies, including breast cancer. Corticosteroids are used to reduce peri-tumoral edema and provide symptomatic relief. Chemotherapy has not generally been useful in the treatment of most epithelial cancers that metastasize to the brain due to the limitations on drug delivery imposed by the blood-brain (or blood-tumor) barrier (see below). Whole brain radiation can provide a median survival of 4 to 5 months, which can be further extended by stereotactic radiosurgery.[15,17] Several nonrandomized studies have suggested that stereotactic radiosurgery may provide nearly equivalent outcomes compared to surgery followed by whole brain irradiation.[15,17] Surgery tends to reduce symptoms quickly and prolong life significantly, with persistent increases in quality of life.[18–20] Multiple metastases (up to three) can be removed surgically with a risk similar to that of a single lesion, providing similar benefits.[3,16] At present, adjuvant radiotherapy follows surgical resection because this combined approach has been shown in general to prolong median survival significantly, to ~12 months depending on the factors noted above.[3,16] There is a growing body of evidence that surgery may be useful in select patients with recurrent brain metastases.[3,16]

Mean survival from diagnosis of a brain metastasis varies between studies but ranges from 2 to 16 months, depending on involvement of the CNS, the extent of the extra-cranial metastatic disease, and the treatment applied. The mean 1-year survival is estimated at ~20%.[16,21] Traditionally, fewer than 2% of patients with breast cancer survive greater than 2 years after the advent of CNS involvement; the inability to control extracranial (systemic) disease has traditionally been the main limiting factor.[1,3,16,21] However, as systemic therapies improve, control of extra-cranial disease may become less influential a predictive factor. This point is strengthened by studies of Her-2-positive patients treated with trastuzumab, a monoclonal antibody against the receptor. In a recent study reported by Bendell and colleagues,[10] the median survival of patients with metastatic breast cancer treated with trastuzumab was 13 months, and nearly half of all patients died as a result of progressive CNS disease.

## *Site-Specific Metastasis Research*

Certain general steps are necessary for metastasis and have been described in a variety of recent reviews.[22,23] These include invasion of the primary tumor border and intravasation of the circulatory system, survival and arrest in the circulation, extravasation to a distant site, formation of a micrometastasis and then progressive colonization to form a life threatening metastasis. Since Paget theorized in 1889[24] that metastasis is ruled by both the "seed" (the tumor cells) and the "soil" (the host), the nature of site-specific metastasis has been pondered.[24,25] Breast cancer principally metastasizes to the regional lymph nodes, bone, liver, lungs, and brain. However, most transgenic and xenograft systems model only a fraction of these sites simultaneously. Thus, we are left wondering how distinct are metastases arising in the soil of the lungs versus the soil of the brain, and what are the therapeutic implications of such differences?

Bone may represent the organ site of breast cancer metastasis in which research has generated the greatest insights.[26,27] Osteolytic metastases appear to be regulated by tumor production of parathyroid hormone-related peptide (PTHrP), which activates osteoblasts and osteoclasts in the bone. Osteoclasts destroy bone matrix, releasing embedded growth factors that further stimulate the tumor cells, creating a vicious cycle. Other bone metastasis pathways include interleukin (IL)-8 and the receptor activator of nuclear factor-$\kappa$B ligand (RANKL) system. Microarray analyses of primary tumors and metastases have yielded conflicting results with as yet uncertain conclusions.[28–31] Using a model system, Kang and colleagues[32] reported that both poorly and highly bone metastatic cell lines lost a 17-gene overall metastatic signature set previously described by Ramaswamy and colleagues.[31] They also found a distinct, differentially-expressed set of bone metastasis genes, including connective tissue growth factor, IL-11, chemokine (C-X-C motif) receptor 4 (CXCR4), and osteopontin, contributed to bone metastatic potential.[32] Taken together, these data suggest that successful metastases have a set of general metastatic competency genes and that tissue-specific gene expression may be necessary to grow in a particular soil.

## *Model Systems*

The ability to form and test hypotheses improves with the availability of relevant model systems. The MDA-MB-435 and -231 human breast carcinoma cell lines have served as the mainstay of brain metastasis work. To our knowledge only one report, using a luciferase-labeled MDA-MB-435 cell line, has identified brain metastases from orthotopic (mammary fat pad, mfp) injection. Both cell lines have produced brain metastases in experimental metastasis assays, via infusion into either the carotid artery or the left cardiac ventricle.

Recently, the laboratories of Zhang and colleagues,[33] Yoneda and colleagues,[34] and Kim and colleagues[35] performed successive rounds of culture of isolated brain metastases and reinjection into animals to produce sublines with enhanced brain metastatic potential and/or increased selectivity for brain compared to other metastatic sites. For MDA-MB-231 cells, six rounds of selection resulted in the MDA-MB-231BR subline that metastasized with 100% frequency to the brain but was undetectable in other organs.[34] The MDA-MB-231BR cells exhibited similar tumorigenicity in the mfp compared to a similarly derived bone-seeking subline, but variations were found in production of parathyroid hormone-related protein and in responsiveness to transforming growth factor (TGF)-$\beta$ and insulin-like growth factor

(IGF)-1 *in vitro*. Three rounds of selection via carotid artery injection performed in the laboratory of Kim and colleagues[35] resulted in the BR1, BR2, and BR3 MDA-MB-231 sublines, which exhibited an increasing incidence of brain metastases (82 to 100% of mice) and decreasing times after injection for mice to became moribund (59 to 41 days). A comparable MDA-MB-231 subline, selected for lung colonization, did not show increased incidence of brain metastasis or shorter survival, indicating that the results were due to specific selection for brain colonization ability. The MDA-MB-231 BR1, BR2, and BR3 sublines also differed from parental cells in microvessel density and aspects of angiogenesis.

Single reports in the literature suggest that other cell lines may be capable of brain metastasis *in vivo*, possibly mimicking the clinical/phenotypic/genetic heterogeneity observed in human cancer. These include a human cell line derived from a brain metastasis (MDA-MB-361), commonly studied lines such as MDA-MB-468, and rarely cited lines such as MA11.[33,36] The arduous work of *in vivo* selection and labeling to facilitate experimentation should be a high research priority. As with bone metastasis, both imaging and histological examination are required to confirm brain metastasis formation since individual labeled cells, potentially dormant, can now be imaged. A rat model of leptomeningeal colonization of Her-2-overexpressing SKBR3 cells was reported, although it requires considerable small animal surgical skills to obtain leptomeningeal metastases in a high percentage of animals.[37] That report, and the carotid artery injections of MDA-MB-231 BR1 to BR3 sublines,[35] demonstrate that certain models are sufficiently robust to provide quantitation of therapeutic effects of compounds in preclinical analyses. It may be possible to use certain models not only for basic molecular biology but for preclinical drug development experiments. These models may prove helpful in gaining a better understanding of drug delivery across the blood-brain, blood-tumor, and blood-CSF barriers, as discussed below. Given the morbidity of certain brain metastasis treatments, it will be of interest to determine whether quality of life can be measured in mouse models, for instance running on a treadmill or wheel, balance, or competency in a maze.

In addition to traditional *in vitro* assays for components of metastasis, including motility, invasion of extracellular matrix, and anchorage-independent colonization, the invasion of human brain microvascular endothelial cells as a model for invasion of the blood-brain barrier (BBB) has been investigated by the laboratory of Lee and colleagues.[38,39] Commercially available human brain microvascular endothelial cells were cultured on plates coated with extracellular matrix; cells were then trypsinized, plated in fibronectin-coated transwell chambers containing 8-$\mu$m pores, and cultured an additional 5 days to establish a BBB. Invasion of labeled MDA-MB-231 cells could be measured relatively quickly (6 hours) by assessing *in vitro* attachment to human brain microvascular endothelial cells, invasion through them, and alterations in endothelial BBB properties (permeability of $^{3}$H-inulin, actin redistribution, and disruption of adherens junction VE-cadherin protein). A second, murine brain capillary endothelial cell line, B.End3, has been reported.[40] Although promising, the *in vitro* BBB lacks significant features of the *in vivo* BBB, including pericytes, astrocytes, and other contributions.

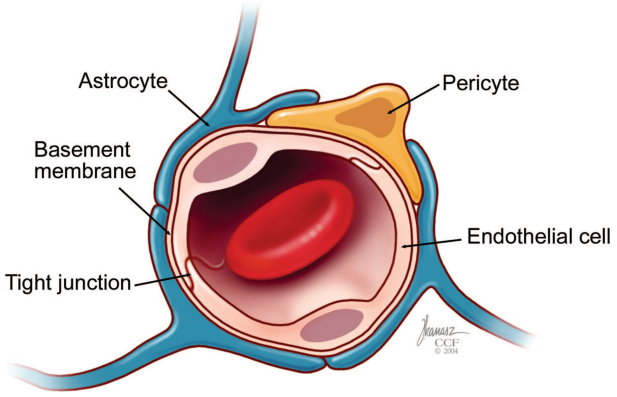


**Figure 2.** Representative mechanistic image of the BBB. The BBB is created by the snug apposition of endothelial cells that line the brain. This creates the barrier between the vascular system and the brain parenchyma. This is reinforced by numerous pericytes. A thin basement membrane surrounds the endothelial cells and provides both structural support and a dense physical barrier between the circulation and the microenvironment of the brain. Commonly, astrocytes extend cellular processes that cover the basement membrane, further limiting the ability of macromolecules or circulating cells to gain access to the CNS. Reprinted with the permission of The Cleveland Clinic Foundation.

## *A Unique Environment?*

The BBB is hypothesized to create and/or interact with the unique brain microenvironment and to influence metastatic colonization. The BBB consists of capillary endothelial cells that lack fenestrations and associate with continuous tight junctions, with a high electrical resistance (Figure 2).[41,42] Pericytes, basement membrane, and the feet of astrocytes line the endothelial cells. Low permeability to ions and small molecules and virtual impermeability to macromolecules and peptides is observed. A lack of pinocytosis, which facilitates the transport of molecules via cellular transcytosis, contributes to selectivity. Both ATP-binding cassette C1 (ABCC1) and ABCB1 (P-glycoprotein) are present on the luminal membrane of the cerebral endothelium, excluding most drugs from entering the brain parenchyma.[43] The BBB works in concert with the blood-CSF barrier to protect the neural environment.

Once tumor cells invade the BBB to establish a brain metastasis, endothelial cells form a blood-tumor barrier (BTB). Almost nothing is known of this barrier in the human or in model systems. One hallmark of brain metastases is the edema that surrounds the tumor, an effect possibly caused by altered permeability of tumor-associated endothelial cells that permits greater leakage of fluid into the tumor.[43] An improved understanding of the interactions between tumor and epithelial cells could assist in the development of new therapeutic approaches.

The brain parenchyma is populated by astrocytes, which can synthesize a host of biologically interesting proteins including IL-1, IL-3, IL-6, interferon-$\gamma$, tumor ne-

crosis factor-$\alpha$, TGF-$\beta$, IGF-1, platelet-derived growth factor-1, and other cytokines.[44] Astrocytes can also serve as antigen-presenting cells for immune responses.[45] Although glial cells have traditionally been thought to provide structural support for neurons, we now know that they also influence brain and BBB integrity.[44,46] When brain metastatic and parental MDA-MB-435 cells were co-cultured with astrocytes or cell culture supernatants of astrocytes, the MDA-MB-435 BR1 cells exhibited increased adherence to astrocytes and better growth in response to the conditioned medium.[44] Therefore, cytokines from the brain microenvironment may provide part of the soil in which the metastatic seed grows.

## *Tissue-Based Studies*

Few studies of human CNS metastases are available because only a small percentage of affected patients undergo surgery. Additionally, many of the traditional cancer molecular markers, as assessed by immunohistochemistry, fail to distinguish primary solid tumors from brain metastases. Two research groups have examined formalin-fixed, paraffin-embedded blocks of matched primary tumors and brain metastases, of which one study included breast carcinomas.[47,48] No significant differences in the expression levels of p53, Bcl-2, E-cadherin, matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, epidermal growth factor (EGF) receptor, cyclooxygenase 2, or Bax were observed between the primary tumors and brain metastases. In contrast, Mehrotra and colleagues[49] identified a DNA hypermethylation phenotype in tissue blocks containing metastatic lesions of breast cancer, including eight brain metastatic lesions. Hypermethylation of *cyclin D2*, *retinoic acid receptor-*$\beta$, and *hin-1* occurred more frequently in brain metastases than in an unmatched cohort of primary breast carcinomas whereas hypermethylation of other genes, including *twist* and *RassF1A,* was not significantly different. DNA hypermethylation was observed in metastases to bone and lung as well. The data suggest that DNA methylation may contribute to altered gene expression in brain and other metastases and suggest the potential relevance of demethylating agents in clinical treatment.

## *The Emerging Her-2 Connection*

Her-2 is a member of the epidermal growth factor receptor superfamily. It is overexpressed in ~20 to 30% of breast carcinomas via gene amplification, and its overexpression correlates with poor patient survival.[9] Her-2 dimerizes with other members of the EGF receptor superfamily to initiate signaling that controls or influences multiple aspects of growth and differentiation. Trastuzumab (Herceptin; Genentech, South San Francisco, CA), is a recombinant, humanized monoclonal antibody to Her-2 that has been reported to extend survival in metastatic breast cancer patients when used as a single agent or in combination with cytotoxic chemotherapy.[50]

Many potential molecular mechanisms have been suggested to mediate the tumor aggressiveness phenotype of Her-2. For example, increased activation of Her-2 signaling has dramatic effects on cell proliferation, survival, apoptosis resistance, migration, and invasion.[51] Bendell and colleagues[10] retrospectively studied 122 women treated with trastuzumab alone or in combination with chemotherapy for Her-2-overexpressing metastatic breast cancer. Based on a median follow-up of 23 months, 34% of patients were diagnosed with CNS metastases, well above historical rates. At the time of diagnosis of CNS metastasis, 50% of patients were responding to therapy or had stable disease. This report was confirmed by the study of Clayton and colleagues,[52] which followed 93 metastatic breast cancer patients. Brain metastases occurred in 25% of patients during a median follow-up period of 10.8 months from the start of trastuzumab therapy. Of 23 patients developing CNS metastases, 78% had stable disease at other sites while on trastuzumab therapy. The CNS was the first site of symptomatic disease progression in 82% of patients and the only site of disease progression at that time in 69% of patients. Both studies report frequencies of brain metastases above those reported for all breast cancer patients in historical studies. Furthermore, CNS metastases tended to occur in patients who were otherwise doing well on trastuzumab therapy.

Another study used a different approach and screened 155 women with metastatic breast cancer, but no symptomatic CNS metastases, before entry into several molecularly-based anti-angiogenic clinical trials.[12] This was unusual because CNS screening is not commonly conducted on asymptomatic patients. However, nearly 15% of the women screened had occult brain metastases, and Her-2 overexpression by the primary tumor was predictive of occult brain metastases. Survival among patients with occult brain metastases was shorter than that of patients without CNS disease but was similar to the survival of patients with symptomatic brain metastases.[12]

The causes of these trends are unknown. One theory suggests that Her-2 overexpression endows tumor cells with increased metastatic aggressiveness to sites such as the lungs and may similarly augment metastatic propensity to the CNS.[53,54] The development of brain metastastic models for breast cancer can permit direct testing of this hypothesis through transfection experiments. Second, by allowing patients to live longer, trastuzumab may allow micrometastatic brain metastases to become symptomatic as a natural consequence of an extended life span. A nonexclusive, third theory posits that trastuzumab is effective against systemic metastases but relatively ineffective against CNS metastases due to its poor penetration of the BTB. This hypothesis may extend to cytotoxic chemotherapy as well as trastuzumab.[5] Limited pharmacokinetic data in support of this hypothesis suggest that systemic administration of trastuzumab results in drug levels in the CSF that are 300-fold lower than in the serum.[55] Also, intrathecal administration of 4D5, the murine precursor of trastuzumab, shows efficacy against a human xenograft of Her-2-overexpressing cancer

growing in the leptomeninges, suggesting that trastuzumab could be efficacious if it could penetrate the BTB.[37] These data also suggest that lipophilic small molecule inhibitors of Her-2 or its dimerization partners may have therapeutic benefit. Finally, Grossi and colleagues[56] used convection-enhanced delivery to administer trastuzumab to intracereberal metastases in an animal model, with encouraging results.

Confounding any clear understanding of these trends, one of the three studies simultaneously reported that hormone receptor-negative primary tumors also significantly correlated with CNS metastasis.[52] Was this the result of reasonable penetration of the BBB by tamoxifen, used as an estrogen receptor antagonist for ER+ cancer? Does this reflect an intrinsically aggressive nature of ER− breast tumor cells? Or is this an epiphenomenon of increased Her-2 overexpression, which is correlated with ER negativity? These remain subjects for experimental inquiry.

## Other Molecular Targets

The potential role of angiogenesis in breast cancer metastasis to the brain has been studied, in particular the role of vascular endothelial growth factor (VEGF), a principle angiogenic factor. When the ZR75-1 human breast cell line was injected either into the mfp or intracranially into nude mice bearing estrogen pellets, the resulting cranial tumors exhibited a higher vascular density than mfp tumors.[57] However, a lower vascular permeability was also observed, suggesting the presence of a proangiogenic, leakage-resistant environment. Two additional studies have suggested a role for VEGF in this process. Lee and colleagues[39] reported that exogenous VEGF increased the penetration of metastatic MDA-MB-231 breast carcinoma cells through a transwell invasion assay containing human brain microvascular endothelial cells. VEGF also modulated the permeability of the endothelial cells. The laboratory of Kim and colleagues[35] reported that the BR2 and BR3 sublines of MDA-MB-231 exhibited increased microvessel densities *in vivo* compared to the parental line. The brain-selective lines also produced higher levels of the angiogenic factors VEGF-A and IL-8 *in vitro* compared to the parent line. In addition, the mean metastatic burden of BR3 cells injected into the carotid artery of nude mice was reduced by 63% by oral administration of PTK787, a VEGF receptor tyrosine kinase inhibitor. PTK787 treatment was associated with fewer microvessels, a decrease in the number of proliferating cell nuclear antigen-staining tumor cells, and greater numbers of apoptotic tumor cells in the experimental brain metastases. These data not only functionally link VEGF to brain metastasis but also demonstrate the potential utility of model systems for preclinical validation studies. It will be of interest to know the impact of this compound when given after symptomatic lesions have formed.

Several molecular determinants of apoptosis have also been studied in model systems. Using the brain metastatic variant of MDA-MB-435 cells, Real and colleagues[58] noted that Bcl-2 expression and Stat3 activation were induced by EGF and contributed to *in vitro* chemoresistance. Rubio and colleagues[59] examined MDA-MB-435 cells that were transfected with the antiapoptotic gene *Bcl-XL*. As assessed by imaging, no brain metastatic lesions were detected 45 days after injection. However, the *Bcl-XL* transfectants were 30-fold more apparent in the brain than in control transfectants in a long-term assay (day 110 after injection), although these trends did not reach statistical significance.

Several cytokines, chemokines, and growth factors have been implicated in brain metastases. Chemokines have been reported to contribute to breast cancer metastasis and may contribute to organ specificity.[60] CXCL12 (stromal cell-derived factor 1a, SDF-1a), a ligand for the CXCR4 chemokine receptor, has been reported to be expressed in brain.[61] Using the *in vitro* invasion assay, Lee and colleagues[38] reported that CXCL12 allowed MDA-MB-231 to invade through human brain microvascular endothelial cells. In other experiments, a brain-homing clone of MDA-MB-231 appeared less responsive to paracrine signals than a comparable bone-seeking clone.[34] When compared to brain-homing tumor cells, bone-seeking cells produced greater levels of PTHrP and plasminogen activator inhibitor 1 (PAI-1), exhibited greater IGF-1R phosphorylation on ligand stimulation, and were resistant to TGF-$\beta$ inhibition of soft agar colonization. The brain-homing cells, however, did demonstrate a moderate stimulation of soft agar colonization by IGF-1. These data may reflect a general insensitivity of brain metastases to endocrine signals, given their poor penetration of the BBB. Alternatively, brain metastases may show exquisite reactivity to distinct signals. Candidates comprise locally produced factors including receptors for neurotrophins such as nerve growth factor, transferrin, gangliosides, and other enzymes.[62–69]

## Conclusions

The CNS is a common sanctuary site of metastatic disease in patients with breast cancer. We predict that brain metastases will become increasingly prevalent as greater control over systemic metastases is achieved, particularly with regard to Her-2-positive tumors. Because of the BBB and the unique microenvironment of the brain, distinct therapeutic approaches for brain metastases may require development. The recent advancement of cell lines capable of experimental metastasis to the brain should facilitate molecular analyses and preclinical development studies. However, with only a few model systems available, principally derived from MDA-MB-231 cells, it is critical to conduct studies on human tissue to assess the generality of the molecular pathways identified. The design of therapeutic approaches to brain metastases would further benefit from an increased understanding of the blood-brain and blood-tumor barriers as well as other host-tumor interactions in the CNS.

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